

AN AQUATIC TOXICOLOGICAL EVALUATION OF SULFATE: THE CASE FOR CONSIDERING HARDNESS AS A MODIFYING FACTOR IN SETTING WATER QUALITY GUIDELINES

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Abstract—Elevated concentrations of sulfate occur commonly in anthropogenically impacted and natural waters. However, water quality guidelines (WQG) have not been developed in many jurisdictions, and chronic toxicity data are scarce for this anion. A variety of test organisms, including species of invertebrate, fish, algae, moss, and an amphibian, were tested for chronic toxicity to develop a robust dataset that could be used to develop WQGs. As an example of how these data might be used to establish guidelines, calculations were performed using two standard procedures: a species sensitivity distribution (SSD) approach, following methods employed in developing Canadian WQGs, and a safety factor approach, according to procedures typically used in the development of provincial WQGs in British Columbia. The interaction of sulfate toxicity and water hardness was evaluated and incorporated into the calculations, resulting in separate values for soft (10–40 mg/L), moderately hard (80–100 mg/L) and hard water (160–250 mg/L). The resulting values were 129, 644, and 725 mg/L sulfate, respectively, following the SSD approach, and 75, 625, and 675 mg/L sulfate, following the safety factor approach. Environ. Toxicol. Chem. 2011;30:247–253. # 2010 SETAC

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INTRODUCTION

Sulfate commonly occurs at elevated concentrations in wastewaters from industrial processes, in runoff from agricultural areas, and in natural waters draining areas of significant mineralization. Federal water quality guidelines (WQGs) have not been developed in Canada or the United States for this anion. However, the province of British Columbia (BC) established a WQG for sulfate of 100 mg/L for the protection of aquatic life in freshwater [1]. The absence of widely applied WQGs, and the presence of sulfate at elevated concentrations in some discharges, suggests a need for establishing a science-based threshold for adverse effects associated with this anion.

The current BC WQG of 100 mg/L is based largely on results from toxicity tests using three test species, representing an invertebrate, a plant, and a fish. In particular, the tests were a 96-h survival test using *Hyalella azteca*, which resulted in a median lethal concentration (LC50) of 205 mg/L sulfate under hardness conditions of 25 mg/L (as CaCO₃); a 21-d growth test using the aquatic moss *Fontinalis antipyretica*, which indicated that adverse effects may occur at 100 mg/L sulfate; and a 96-h survival test using larval striped bass (*Morone saxatilis*), which yielded an LC50 of 250 mg/L [1].

Results of these tests have been questioned in the literature, in part because of the inability of researchers to replicate them. For example, Davies [2] reported statistically detectable effects

on growth and chlorophyll content of *F. antipyretica* at 400 mg/L, but not at 200 mg/L sulfate. These authors suggested that the higher degree of toxicity observed by Frahm [3], and incorporated in the BC WQG [1], likely related to the use of potassium sulfate in that study, rather than sodium sulfate. Potassium exhibits a greater degree of toxicity as compared with Na [4] and, consequently, is not suitable as a counter-ion in any evaluations of the toxicity of anions.

The sensitive LC50 value of 205 mg/L sulfate for *H. azteca* in very soft water, which was taken from an unpublished study conducted by Environment Canada (Pacific Environmental Science Centre, North Vancouver, BC, Canada) [1], was questioned by Davies et al. [5], who obtained an LC50 value of 491 mg/L sulfate in a test with this species under similar hardness conditions (25 mg/L). Davies et al. also observed poor survival in control exposures from a number of tests conducted at this hardness, suggesting that the water quality conditions associated with the low-hardness water may have caused stress to the test organisms.

Finally, data characterizing sulfate toxicity to larval striped bass reported by Hughes [6], and incorporated into the BC guideline [1], have also been questioned [5]. This species is anadromous, spawning in the lower reaches of rivers, including estuaries. The optimal salinity for larval development in this species is 10 ppt seawater, which would contain approximately 775 mg/L sulfate. Thus, the LC50 value of 250 mg/L reported for sulfate is not supported; this result suggests an effect of ionic composition of the test water, rather than of sulfate toxicity. Indeed, Davies et al. [5] reported improved survival of this species with increasing sulfate concentrations, with the highest rate of survival at the highest sulfate concentration tested (4,000 mg/L);

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these authors postulated that the high degree of sensitivity observed by Hughes [6] was related to osmotic stress associated with use of a low-ionic-strength dilution water in that study.

A growing body of evidence suggests that the toxicity of sulfate decreases with increasing hardness. This effect has been demonstrated in acute toxicity studies using rainbow trout, coho salmon, *H. azteca*, and *Daphnia magna* [1,7,8], as well as in sublethal tests, including embryo-development tests with rainbow trout and survival and reproduction tests using *D. magna* [1]. The mechanism by which ions constituting water hardness influence the toxicity of sulfate has not yet been established; however, this phenomenon likely relates to either competition from other ions at ionic uptake sites in the gill, or an effect on membrane permeability, as suggested by Penttinen et al. [9]. Reduced toxicity of a major anion caused by higher water hardness also has been reported for another major anion, chloride, in sublethal toxicity tests using *Ceriodaphnia dubia* [10].

Based on the apparent importance of water hardness for sulfate toxicity and the concern over the robustness of key data employed to derive the BC WQG for protection of aquatic life, additional effort appears warranted to establish safe, scientifically defensible levels for sulfate in the aquatic environment. Consequently, the present study was conducted to provide a comprehensive set of toxicological data, generated using standardized test methods, which can be used to establish safe limits for sulfate under varying conditions of hardness.

Test species and types were selected to meet Canadian Council of Ministers of the Environment requirements for establishing Type A Canadian WQGs for the protection of aquatic life, using a Species Sensitivity Distribution (SSD) approach [11]. This process requires toxicity test data of suitable quality for three fish species (including at least one salmonid and one non-salmonid); three aquatic invertebrates (including at least one planktonic crustacean); at least one vascular plant or freshwater alga; and aquatic life stages of an amphibian (considered highly desirable, although not required).

MATERIALS AND METHODS

Test species, durations, and endpoints were chosen on the basis of providing a suitable representation of species to meet Canadian Council of Ministers of the Environment requirements [11] for which chronic exposures could be conducted in the laboratory following standardized published procedures. Species selection also accommodated selection of species that have previously been demonstrated to be sensitive to sulfate (*H. azteca*, *F. antipyretica* [1]).

Test organisms included three species of invertebrates (a cladoceran [*C. dubia*], a rotifer [*Brachionus calyciflorus*], and an amphipod [*H. azteca*]), three species of fish (rainbow trout

[*Oncorhynchus mykiss*], coho salmon [*Oncorhynchus kisutch*], and fathead minnow [*Pimephales promelas*]), one amphibian species (Pacific tree frog [*Pseudacris regilla*]), and two plant species (a green alga [*Pseudokirchneriella subcapitata*] and an aquatic moss [*F. antipyretica*]). All tests were conducted according to standardized biological test methods, with the exception of the tests using *F. antipyretica*, which were based on procedures described by Davies [2], and methods for tests using Pacific tree frogs, which were adapted from the Organisation for Economic Co-operation and Development (OECD) guidance for measuring effects of thyroid-active substances on amphibians [12]. Test durations and endpoints are provided in Table 1.

Exposures were conducted in constant environment rooms that maintained temperature within 18°C of the target temperature. Water used in the tests was dechlorinated municipal tap water (hardness of approximately 15 mg/L), or was prepared by supplementing de-ionized or dechlorinated municipal water with reagent-grade salts according to procedures specified in U.S. Environmental Protection Agency guidance documents to obtain soft, moderately hard, hard, or very hard water [13], with the exception of tests using *H. azteca*, which used a recipe for salt addition that resulted in a higher chloride concentration [14]. The dilution water for the *C. dubia*, *B. calyciflorus*, and *P. subcapitata* tests was deionized water prepared with added salts, and for the remaining species was dechlorinated municipal tap water, with or without the addition of salts. Hardness values of test exposure solutions used for each species are provided in Table 1.

Exposure solutions incorporated five to eight test concentrations, in addition to the control, and followed a 0.5- or a 0.67-fold dilution series, and were prepared by addition of sodium sulfate to achieve the target sulfate concentrations. Sodium sulfate was used rather than the sulfate salts of other cations (Ca, Mg, or K) because Na is expected to contribute the least to toxicity relative to the other cations [4]. Subsamples were collected from the test solutions at the beginning and end of each of the tests, and sulfate concentrations were confirmed analytically using automated colorimetry. Quality assurance/quality control procedures for analytical confirmations included evaluation of sulfate in laboratory duplicate samples and blanks, as well as in sulfate-spiked laboratory water and sample matrix. Quality control limits were 20% relative percent difference for duplicates, 75 to 125% recovery for matrix spikes, and 80 to 120% for laboratory water spikes. The mean measured concentration of sulfate was used to calculate the test endpoints.

Cladocerans

Chronic toxicity tests using *C. dubia* were conducted according to Environment Canada procedures [15] in 20-ml volumes in 25-ml glass test tubes. Exposures of each concentration

Table 1. Summary of toxicity tests used in the aquatic toxicological evaluation of sulfate

Test species	Test duration	Test endpoint(s)	Hardnesses tested (mg/L)
<i>Ceriodaphnia dubia</i>	7–1 d	Survival, reproduction	40, 80, 160, 320
<i>Brachionus calyciflorus</i>	48 h	Population growth	40, 80, 160, 320
<i>Hyalella azteca</i>	96 h	Survival	25, 80
	14 d	Survival, growth	80
<i>Oncorhynchus kisutch</i>	10 d	Embryo development	15
<i>Oncorhynchus mykiss</i>	31 d	Embryo/alevin development	15
<i>Pimephales promelas</i>	7 d	Survival, growth	40, 80, 160, 320
<i>Pseudacris regilla</i>	21 d	Survival, growth	15, 80
<i>Pseudokirchneriella subcapitata</i>	72 h	Population growth	10, 80, 320
<i>Fontinalis antipyretica</i>	21 d	Growth, chlorophyll	15

comprised 10 replicates, each initiated with a single less-than-24-h-old daphnid obtained from in-house cultures. The organisms were cultured at the corresponding test hardness for at least two generations before initiation of the tests. Solutions were renewed with freshly prepared solutions daily, at which time they were fed a mixture of *P. subcapitata* cells and digested yeast, Cerophyl, and trout chow. Exposures were conducted at 25°C under a 16:8 h light:dark photoperiod. Survival and reproductive output were recorded daily for the 7–1-d duration of the tests. Exposures were terminated on the day that at least 60% of the control organisms produced their third brood. Acceptable limits for control performance were 80% or higher survival and production of at least 15 offspring by surviving control organisms producing three broods.

Rotifers

Brachionus calyciflorus tests were conducted under static conditions for 48 h in a culture plate using a 0.5-ml exposure volume and eight replicates per concentration, each containing one rotifer [16]. The test was initiated with organisms that were less than 4 h old, and solutions were supplemented with *P. subcapitata* as food at test initiation. Exposures were conducted at 25°C in the dark. Despite its relatively short duration, this test is considered a chronic test because of the short life history of this organism, and the fact that the method incorporated a reproductive endpoint within this timeframe. The acceptable limit for control performance was a mean intrinsic rate of population increase of ≥ 0.7 .

Amphipods

Acute (96-h) and chronic (14-d) toxicity tests using *H. azteca* were conducted using amphipods obtained from Aquatic Biosystems (Fort Collins, CO). Chronic toxicity tests with this species were conducted using clean sediment comprising beach-collected sand, rinsed with laboratory control water, and supplemented with peat at a rate of 2% by weight. Test methods were modified from Environment Canada procedures that are typically employed to evaluate sediment toxicity [17], by incorporating test solution renewal three times per week throughout exposure with freshly prepared, sulfate-spiked water, at which time yeast, Cerophyl, and trout chow were added as food. These tests were conducted using four replicates per concentration in glass jars containing 100 ml control sediment and filled to 275 ml with the test solutions. The exposures were conducted at 23°C with a 16:8 h light:dark photoperiod. Surviving amphipods were dried at the end of the test on preweighed aluminum weighboats, and dry weight was determined. Acute exposures using this species were conducted in 200-ml volumes in glass jars with no solution renewal and feeding at test initiation and after 48 h exposure [17]. Both acute and chronic exposures were conducted using 10 test organisms per replicate. Acceptable limits for control performance were 90% or higher survival in the acute exposures, 90% or higher survival, and at least 0.1 mg average dry weight per organism in chronic exposures.

Fish (salmonid)

Toxicity tests with rainbow trout and coho salmon were conducted according to procedures described by Environment Canada for early life stages of salmonids [18]. Tests were initiated less than 30 min after dry fertilization of the eggs; gametes for these tests were obtained from the Fraser Valley Trout Hatchery (Abbotsford, BC, Canada) and the Capilano Hatchery (North Vancouver, BC, Canada), respectively. Rain-

bow trout and coho salmon were each exposed using four replicates of 30 embryos in 2-L volumes; coho embryos were exposed at 11°C for 10 d, and rainbow trout were exposed at 14°C for 31 d. Solutions were renewed daily and gently aerated throughout exposure. The endpoint for the coho salmon test (embryo development) was normal embryonic development and for the rainbow trout test (embryo-alevin development) was normal, hatched psh. Acceptable limits for control performance were at least 65% viable alevins in the rainbow trout test and at least 70% normally developed embryos in the coho test.

Fish (non-salmonid)

Fathead minnow tests were conducted according to procedures described by Environment Canada [19], involving a 7-d exposure initiated with less than 24 h post-hatch psh. Larval psh were obtained from Aquatic Biosystems. Tests were conducted using three replicates in 300-ml glass jars containing 250 ml solution and 10 psh per replicate and were exposed at 25°C and under a 16:8 h light:dark photoperiod. Fish were fed with *Artemia salina* nauplii, and solutions were renewed daily throughout the exposure period. At the end of the test, surviving larvae were dried overnight on preweighed aluminum pans, and then weighed. Endpoints from the test were survival and biomass, and acceptable control performance limits were at least 80% normal surviving psh and at least 0.25 mg dry weight per surviving psh.

Amphibian

Tests using *Pseudacris regilla* were conducted according to methods adapted from the Organisation for Economic Co-operation and Development guidance for tests using the frog species *Xenopus laevis* [12]. Tests were initiated with tadpoles (Gosner stage 29) hatched from eggs that were field-collected from a pond near Squamish, BC, Canada. The tests were conducted using three replicates with pve tadpoles in each replicate. Test containers were glass jars containing 1 L test solution, which was aerated throughout the test. Exposures were conducted at 23°C under a 16:8 h light:dark photoperiod. The test organisms were fed daily with Sera Micron. Test endpoints included survival and wet weight, and criteria for acceptable control performance included survival of at least 90%.

Algae

Toxicity tests using *P. subcapitata* were conducted according to procedures described by Environment Canada [20]. Tests were conducted at 24°C under continuous light with intensity of 4,000–400 lux. Exposures were performed in 96-well U-shaped microplates and were initiated with a density of 10,000 cells/ml, obtained from an in-house culture in exponential growth phase. Four replicates were used for the test solutions. Density of algal cells at the end of the 72-h exposure period was measured, using a hemacytometer, and the endpoint from the test was calculated on the basis of cell yield (increase in cell density). Acceptable control performance was based on achieving at least a 16-fold increase in cell density, with a coefficient of variation of 20% or less.

Moss

Toxicity testing using *F. antipyretica* followed procedures described by Davies [2]. Tests were conducted in 300-ml glass jars containing 150 ml test solution and pve 2-cm apical tips in each of four replicates. Test solutions were renewed at 7-d intervals throughout the exposure period. The exposures were conducted at 14°C under continuous light of 1,300 to 1,800 lux.

Test endpoints were length, dry weight, and chlorophyll content. Dry weight was measured on three tips at test termination, after drying for 24 h on preweighed aluminum pans; two tips (the longest and shortest from each replicate) were pooled and used for the measurement of chlorophylls A and B. Chlorophyll measurements were conducted using spectrophotometry after digestion in acetone. Tests using this species were conducted on two occasions, using moss collected from Hazeltine and Musqueam Creeks (BC, Canada). Quantitative criteria for acceptable control performance have not been established for this test; consequently, the test was considered acceptable if the moss tips in the control exposures appeared to be healthy.

Statistical analyses

Statistical analyses were conducted using CETIS (Tidepool Software) and according to procedures described by Environment Canada [21] for calculating point estimates and hypothesis tests. Survival and normality data were analyzed using probit or logit multiple linear estimation, where possible, and quantitative data for reproduction and growth were analyzed using nonlinear regression in cases in which model assumptions were met. Linear interpolation of log-transformed data was used in cases in which the assumptions of the models described were not met. Species sensitivity distributions were calculated using CETIS by multiple linear estimation regression. Log-logit, log-normal (Probit), log-Gompertz, and log-angle models were tested, and the best fit was selected on the basis of the lowest Akaike information criterion value, which provides a relative measure of goodness of fit of various models.

RESULTS AND DISCUSSION

The results of all toxicity tests reported herein met requirements specified in each of the test methods for control performance, and water quality parameters remained within the ranges specified in the corresponding test methods. Quality control limits for analytical chemistry were achieved for duplicate measurements, spikes, and blanks. Measured concentrations of sulfate were consistent with nominal values (Table 2) and showed minimal departure between test initiation and termination; final values were 101–0.9% of initial values in the tests. Presence of sediment in the test using *H. azteca* did not appear to influence the water column concentration of sulfate.

The geometric means of LC50 values for *H. azteca* were 619 and 2,099 mg/L sulfate for tests at 25 mg/L ($n=5$) and 80 to 100 mg/L hardness ($n=6$), respectively, demonstrating a 3.4-fold change in toxicity associated with a similar degree change in hardness. These values are similar to those reported by Davies and Hall [7], who reported LC50 values of 569 and

1,580 mg/L sulfate at hardness of 25 and 75 mg/L, respectively. The LC50 values for water with a hardness of 25 mg/L were more than twofold higher than the 205-mg/L value used in the derivation of the current BC WQG for sulfate [1]. This difference may be related to differences in dilution water used in the tests; for example, chloride has been noted to affect the toxicity of sulfate to *H. azteca* [8].

Results of sublethal toxicity tests conducted during the current study are summarized in Table 3; point estimates are provided for a 10%, 25%, and 50% effect relative to the control. Procedures for developing Canadian WQGs indicate that the most appropriate effect concentration or inhibition concentration values reflecting the toxicological threshold from the test should be used for calculating SSDs, where available [11]. Ideally, tenth percentile effect levels are preferred; however, interpreting the significance of tenth percentile data requires caution in some cases, because the uncertainty of statistical calculation increases substantially in the tails of the distribution, and long-term laboratory toxicity tests rarely have the statistical sensitivity to detect a 10% departure from control performance. The approach taken here involves the use of the 10% inhibition concentration or 10% effect concentration values as the toxicological threshold in cases in which this value exceeded the no observed effect concentration from the test. In cases in which the tenth percentile point estimate was lower than the no observed effect concentration, the toxicological threshold was considered to be the 25th percentile effect levels (25% inhibition concentration [IC25] or 25% effect concentration) from the test, because the dataset was not considered to be sufficiently robust to derive a 10% inhibition concentration value with a suitable degree of confidence.

As observed in acute tests using *H. azteca*, sublethal tests conducted at differing hardnesses also indicated decreasing sulfate toxicity with increasing water hardness for most of the species tested in the current study. For example, biomass of fathead minnows and reproduction of *C. dubia* exhibited an approximate fourfold change in sensitivity to sulfate across a fourfold change in hardness (from 40–160 mg/L hardness) (Fig. 1).

Exceptions to this general pattern were *P. subcapitata* (algae), *B. calyciflorus* (rotifer), and *P. regilla* (amphibian), which exhibited minimal changes in sensitivity to sulfate with increasing hardness. This difference may reflect physiological differences among organisms. For example, algae accumulate sulfate into their cells by active transport, given that sulfate is required by the cells to produce sulfur-containing amino acids, such as methionine and cysteine [22]. Thus, uptake of sulfate by these cells is governed by Michaelis-Menten kinetics, in which the number of active uptake sites limits the maximal uptake rate, and relative concentrations of other ions would not be expected to interfere with uptake kinetics unless they compete for binding sites. Conversely, freshwater fish apparently have no active mechanism for sulfate uptake or regulation at the gill, and sulfate uptake likely occurs by passive diffusion through ion channels. Decreased toxicity of sulfate to these organisms with increasing ionic strength therefore may occur as a result of competitive exclusion by other ions in these channels, or effects of calcium on cell membrane permeability [9].

Whether amphibians have active uptake sites for sulfate is not known, but that may explain why *P. regilla* was not substantially more sensitive to sulfate under soft water conditions. Interestingly, growth of *P. regilla* tadpoles was significantly enhanced in nonlethal concentrations of sulfate; exposure to 1,000 mg/L sulfate produced tadpoles that were approximately 30% heavier than control tadpoles under both

Table 2. Measured concentrations of sulfate as a percentage of nominal in test solutions^a

Species	Measured sulfate as a percentage of nominal (mean SD)	
<i>Ceriodaphnia dubia</i>	104	22
<i>Brachionus calyciflorus</i>	87	7
<i>Hyalella azteca</i>	99	22
<i>Oncorhynchus kisutch</i>	91	6
<i>Oncorhynchus mykiss</i>	93	9
<i>Pimephales promelas</i>	97	14
<i>Pseudocris regilla</i>	97	3
<i>Pseudokirchneriella subcapitata</i>	97	3
<i>Fontinalis antipyretica</i>	102	10

^a Values are presented as a mean and standard deviation for measurements conducted in tests with each species.

Table 3. Responses of various aquatic organisms to waterborne sulfate, at different hardness levels^a

Species	Endpoint	Hardness	EC10 or IC10 ^b	EC25 or IC25	EC50 or IC50	NOEC	LOEC
<i>Ceriodaphnia dubia</i>	Survival	40	NC ^c	NC	914 (809–1,030)	610	1,300
		80	NC	NC	1,267 (1,026–1,566)	1,250	2,000
		160	NC	NC	1,551 (1,297–1,855)	1,300	2,600
		320	NC	NC	1,619 (1,364–1,920)	1,450	2,700
	Reproduction	40	137 (71–204)	246 (172–326)	465 (358–592)	<150	150
		80	622 (NC–813)	855 (601–1,036)	1,129 (990–1,269)	645	1,250
		160	1,174 (1,153–1,188)	1,212 (1,206–1,219)	1,257 (1,248–1,267)	775	1,300
		320	402 (331–481)	542 (455–640)	843 (710–1,003)	420	480
<i>Brachionus calyciflorus</i>	Reproduction	40	703 (158–1,013)	997 (739–1,115)	1,214 (1,083–1,308)	950	1,800
		80	245 (148–744)	1,824 (721–1,921)	2,200 (2,089–2,277)	510	960
		160	678 (258–1,059)	1,292 (1,078–1,766)	>1,800	560	1,100
		320	844 (795–1,174)	1,027 (900–NC)	>1,800	1,800	>1,800
<i>Hyalella azteca</i>	Survival	80	2069 (NC)	2,246 (NC)	2461 (NC)	1,637	2,412
	Reproduction	80	380 (NC–626)	1,056 (NC)	>2,412	1,637	2,412
<i>Oncorhynchus kisutch</i>	Embryo	15	941 (803–1,062)	1,264 (1,128–1,391)	1,755 (1,607–1,921)	825	1,450
<i>Oncorhynchus mykiss</i>	Embryo-alevin	15	356 (256–433)	501 (407–582)	734 (640–823)	205	340
<i>Pimephales promelas</i>	Survival	40	559 (293–805)	933 (601–1,230)	1,649 (1,255–2,097)	595	1,250
		80	1,555 (869–2,032)	2,183 (1,499–2,634)	2,938 (2,359–3,385)	1,300	2,850
		160	3,231 (2,084–3,840)	3,801 (2,817–4,356)	4,553 (3,827–5,157)	2,850	5,500
		320	2,451 (1,129–> 5,250)	>5,250	>5,250	2,900	5,250
	Biomass	40	388 (187–553)	752 (537–943)	1,244 (1,047–1,449)	595	1,250
		80	1,342 (NC–1,926)	1,950 (915–2,485)	2,591 (2,211–2,975)	760	1,300
		160	2,491 (NC–2,934)	3,077 (2,525–3,541)	3,892 (3445–4397)	1,300	2,850
		320	1,323 (297–2,656)	3,463 (1,953–5,473)	>5,250	820	1,400
<i>Pseudacris regilla</i>	Survival	15	719 (234–1,041)	1,190 (750–2,002)	>1,850	978	1,850
		80	985 (146–1,302)	1,205 (363–1,510)	1,507 (907–1,963)	1,075	1,925
	Growth	15	1,342 (NC–1,905)	1,560 (NC–2,079)	1,853 (1,646–2,082)	978	1,950
		80	1,252 (NC–1,492)	1,348 (NC–1,649)	1,510 (1,217–2,167)	1,075	1,925
<i>Pseudokirchneriella subcapitata</i>	Cell yield	10	700 (NC–1,256)	1,112 (262–1,325)	1,430 (1,206–1,637)	1,100	2,000
		80	1,345 (NC–1,532)	1,763 (936–2,170)	2,742 (1,871–3,221)	1,200	2,700
<i>Fontinalis antipyretica</i>	Chlorophyll	320	1,377 (NC–1,582)	1,727 (1,371–1,983)	2,518 (2,093–3,007)	1,300	2,800
		15 (test 1)	53 (NC–261)	176 (NC–320)	298 (158–1,014)	145	300
	Growth	15 (test 2)	716 (680–750)	820 (777–920)	1,029 (931–1,288)	654	1,240
		15 (test 1)	531 (243–818)	849 (600–1,034)	>2,575	603	1,250
		15 (test 2)	297 (NC–1,025)	828 (335–1,040)	>2,522	654	1,240

^a Responses are presented on the basis of 10th, 25th and 50th percentile effect concentration (ECx) or inhibition concentration (ICx), as well as no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC).

^b IC10 and EC10 values were not considered sufficiently robust for use in guideline calculation in cases in which they were lower than the NOEC.

^c NC = Not calculable.

hardness regimens tested. Thus, tadpoles of this species may have an active uptake mechanism for sulfate, and this anion may be utilized beneficially in metabolic processes.

At the highest hardness tested (320 mg/L), a continued reduction in sulfate toxicity to fathead minnows was observed relative to tests conducted at lower hardness values. However,

C. dubia exhibited increased sensitivity to sulfate in this water relative to tests at a hardness of 160 mg/L. Likely the overall ionic strength of the test solutions in this very hard water type resulted in an osmotic challenge to this species that, combined with elevated sulfate concentrations, resulted in the adverse effect. This relationship also has been reported for chloride [10],

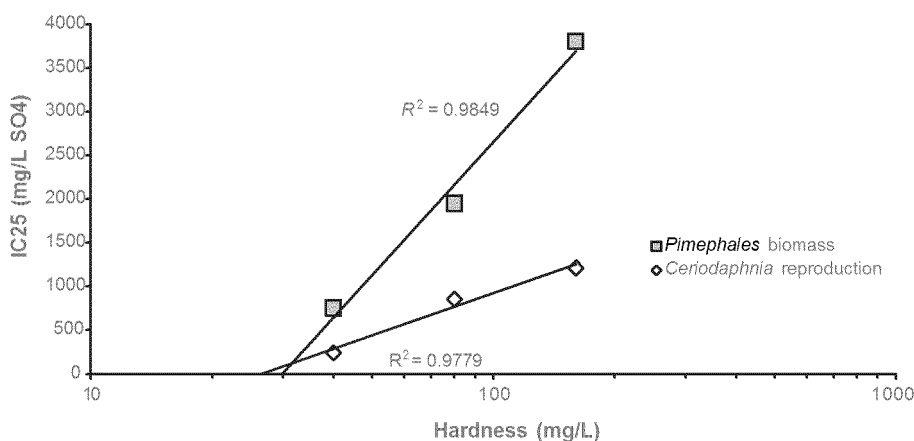
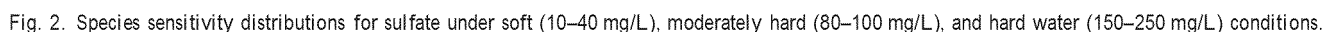


Fig. 1. Relationship between sulfate toxicity, presented as the 25th percentile inhibition concentration (IC25), and water hardness for toxicity tests with fathead minnows (*Pimephales promelas*) and *Ceriodaphnia dubia* across a range of hardnesses from 40 to 160 mg/L.



The current BC WQG for protection of aquatic life [1] was calculated based largely on the application of a twofold safety margin to results from an unpublished acute toxicity test result for *H. azteca* of 205 mg/L, resulting in a guideline of 100 mg/L [1]. This value also took into consideration data for an aquatic moss (*F. antipyretica*) and larval striped bass (*M. saxatilis*). Conversely, the results presented in this study demonstrate that *H. azteca* is not the most sensitive species; daphnids were the most sensitive species tested in each of the hardness ranges, with IC25 values of 245 mg/L (*C. dubia*), 833 mg/L (*D. magna*; data from Singleton [1]), and 1,213 mg/L (*C. dubia*) at hardnesses of 25, 80, and 160 mg/L, respectively. In developing WQGs, the BC Ministry of Environment typically applies between a twofold and 10-fold safety margin below the lowest available lowest observed effect concentration; selection of a safety margin depends on the quality and quantity of available data, severity of adverse effects, and the potential for bioaccumulation [23]. In the case of this dataset, in which sublethal toxicity data are available for a number of sensitive species, a twofold safety margin appears to be appropriate; this approach is consistent with the current BC water quality guideline for this

Tests conducted in hard water, arbitrarily grouped as tests conducted in waters of between 160 and 250 mg/L, were available for two cladocerans, a rotifer, and two species of fish. The hazard coefficient associated with the 5th percentile of the SSD calculated from this dataset (log-angle model) was 725 mg/L sulfate, which provides a suitable level of protection below the most sensitive IC₂₅ of 1,213 mg/L for *C. dubia*, and is

similar to the guideline of 650 mg/L calculated using the BC approach.

CONCLUSION

Development of WQGs requires a robust dataset of toxicological thresholds representing a broad range of sensitive test organisms representing those that occur in receiving water bodies. The battery of tests needs to include sublethal exposures to ensure that resulting guidelines are suitably protective. Data should be of primary quality, which requires that the tests be conducted according to standardized procedures; analytical confirmation of actual concentrations to which test organisms are exposed; and the tests meet quality control requirements for health and sensitivity.

The data presented herein provide a dataset that, we propose, meet these requirements. Furthermore, the data demonstrate that sulfate toxicity is dependent on concentrations of other major ions, with a general reduction in toxicity associated with an increase in water hardness. Thus, accommodating hardness as a toxicity-modifying factor appears to be appropriate in establishing water quality guidelines for this anion. Calculation of guidelines for soft, moderately hard, and hard water conditions using the BC and Canadian Council of Ministers of the Environment approaches for development of WQGs resulted in generally similar results, although under soft water conditions, the BC approach provided a guideline that was approximately twofold more conservative than the Canadian Council of Ministers of the Environment approach.

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